PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 June 2001 (22.06.01)	
International application No. PCT/IB00/00966	**Applicant's or agent's file reference** INV0978
International filing date (day/month/year) 14 July 2000 (14.07.00)	**Priority date** (day/month/year) 20 July 1999 (20.07.99)
Applicant VITALONE, Vincenzo et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 February 2001 (09.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Olivia TEFY

Telephone No.: (41-22) 338.83.38

14

PATENT COOPERATION TREATY

REC'D 07 NOV 2001

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
INV0978		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB00/00966	14/07/2000	20/07/1999
International Patent Classification (IPC) or national classification and IPC G01N33/48		
Applicant AZIENDA PROVINCIALE PER I SERVIZI SANITARI et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/02/2001	05.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Klee, B Telephone No. +49 89 2399 2675

Form PCT/IPEA/409 (cover sheet) (January 1994)

render the determination of benzoylecgonine a necessary and sufficient condition for the detection of cocaine.

Benzoylecgonine is found in the hair in a ratio of 1 to 4 or even 1 to 10 and more with respect to cocaine. Therefore, when the amount of cocaine is very low or close to the cut-off values (0.2-0.1 ng cocaine per mg hair) screening cannot be performed because of the low amount of this metabolyte (0.05-0.025 ng benzoylecgonine per mg hair), such amount being undetectable by the screening apparatuses available in the laboratories.

US-A-5 910 419 which is the nearest prior art to the invention relates to a method for screening hair samples using the well known ELISA technique for the presence of cannabinoids and further RIA for the presence of cocaine. As already outlined, the radio immunoassay does not lend itself to quick and cheap determination of drug of abuse and further involves safety and handling difficulties.

Summary of the invention

The main object of this invention is to overcome the above mentioned drawbacks, i.e. the impossibility to dose the cocaine present in a solid sample by the conventional screening-type approach.

In accordance with the invention, this object is achieved by a screening-type procedure for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which, in accordance with claim 1, includes the following steps:

a) preparing a solid sample in a finely divided or powdered form;

b) selecting a liquid reagent providing constant concentration of

hydroxyl groups suitable for extracting and transforming cocaine into benzoylecgonine and for extracting other similar substances;

c) extracting cocaine and other similar substances contained in the sample and transforming the extracted cocaine into benzoylecgonine by maintaining the sample completely immersed in said liquid reagent at a temperature ranging from 10°C to 250°C for a period of time ranging from few seconds to 48 hours; and

d) analysing the liquid separated from the solid sample to determine the concentration of benzoylecgonine contained in said liquid with respect to the cut-off limit using a conventional screening kit for the determination of the said substance in urine.

Preferred aspects of the invention include using hair as the solid material forming the solid sample; using a buffer as the reagent which provides hydroxyl groups, more preferably an ammonia buffer, most preferably a buffer that is hereinafter referred to as VMA; and heating the sample immersed in the liquid at a temperature of about 100 to 150°C for about one hour.

In accordance with another aspect of the invention a process is provided which includes the additional steps of:

- arranging samples by increasing concentrations of the substances of interest; and
- performing confirmation analyses with known techniques, such as, GC or GC/MS.

In one embodiment of the invention, the process may provide the following steps:

- providing a sample made of about 50 to 300 mg of finely divided and/or powdered material;

- adding in the test tube containing the said sample a suitable liquid reagent until the sample is completely immersed, said reagent being capable of performing extraction and transformation of cocaine into benzoylecgonine and at the same time of extracting other similar substances which are present in the sample
- if necessary, agitating the test tube to facilitate immersion of the sample;
- heating the contents of the test tube to a temperature T1 for a time interval t1 by keeping the test tube immersed in a thermostated bath or by placing it in an oven;
- cooling the test tube to room temperature;
- taking the liquid and transferring it into a test tube suitable for a screening type instrument;
- performing the screening by using a kit of reagents for the determination of the said substances in urine;
- reading the data resulting from the first level instrumentation to verify the concentration values with respect to the cut-off limit; and

AMENDED SHEET

AMENDED CLAIMS

1. A screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which includes the steps of:

a) preparing a solid sample in a finely divided or powdered form;

b) selecting a liquid reagent providing constant concentration of hydroxyl groups suitable for extracting and transforming cocaine into benzoylecgonine and for extracting other similar substances;

c) extracting cocaine and other similar substances contained in the sample and transforming the extracted cocaine into benzoylecgonine by maintaining the sample completely immersed in said liquid reagent at a temperature ranging from 10°C to 250°C for a period of time ranging from few seconds to 48 hours; and

d) analysing the liquid separated from the solid sample to determine the concentration of benzoylecgonine contained in said liquid with respect to the cut-off limit using a conventional screening kit for the determination of the said substance in urine.

2. Process according to claims 1, wherein said solid sample is a sample of hair.

3. Process according to claims 1 and 2, wherein said temperature is ranging from 100°C to 150°C.

4. Process according to claims 1 and 2, wherein said period of time is ranging from 15 minutes to 24 hours.

5. Process according to any preceding claims, wherein said temperature is maintained at 100°C for 1 hour.

AMENDED SHEET

6. Process according to claim 1, wherein said liquid reagent is an ammonia buffer comprising 0.2 M (NH4)2HPO4 with the addition of 5 ml of 25% NH4OH to each liter thereof.

7. Process according to claim 6, wherein the concentration of hydroxyl groups in said ammonia buffer is in the range of from 0.0001M to 5 M.

8. Process according to claim 6, wherein the concentration of hydroxyl groups in said ammonia buffer is in the range of 0.03M to 0.5 M.

9. Process according to claim 6, wherein the concentration of hydroxyl groups in said ammonia buffer is in the range of 0.04M to 0.33 M.

10. Process according to any preceding claims, wherein the analyzed samples are arranged in increasing order of concentration of cocaine or other alkaloids.

11. Process according to claim any preceding claims, wherein the samples are subjected to confirmation analyses with standard techniques such as GC or GC/MS.

12. Process according to any preceding claims, wherein each hair sample is made of about 50mg to 300 mg of finely divided and/or powdered hair.

13. Process according to claim 1, wherein said liquid reagent is a solution comprising a solute selected among aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide,potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate,

AMENDED SHEET

tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, dissolved in a solvent selected among ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

14. Diagnostic kit for the carrying out of the process according to any claims 1 to 13, comprising a liquid reagent with constant concentration of hydroxyl groups suitable for extracting cocaine and other alkaloids and transforming cocaine into benzoylecgonine, and a conventional screening kit for the determination of said metabolite in urine samples.

AMENDED SHEET

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB00/00966

I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-4,7-20	as originally filed			
5,6,6a	as received on	15/10/2001	with letter of	10/10/2001

Claims, No.:

1-14	as received on	15/10/2001	with letter of	10/10/2001

Drawings, sheets:

1/16-16/16	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 14.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 14 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. R asoned statement under Article 35(2) with r gard to novelty, inventive step r industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB00/00966

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
see separate sheet

1. References cited:
   D1: US-A-5 910 419
   D2: SEGURA J ET AL: 'Immunological screening of drugs of abuse and gas chromatographic-mass spectrometric confirmation of opiates and cocaine in hair' JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS,NL,ELSEVIER SCIENCE PUBLISHERS, vol. 724, no. 1, 5 March 1999 (1999-03-05), pages 9-21
   D3: WO 93 03368 A (PSYCHEMEDICS CORP) 18 February 1993 (1993-02-18)
   D4: TAGLIARO F ET AL: 'Hair analysis, a novel tool in forensic and biomedical sciences: new chromatographic and electrophoretic/electrokinetic analytical strategies' JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL SCIENCES & APPLICATIONS,NL,ELSEVIER SCIENCE PUBLISHERS, vol. 689, no. 1, 7 February 1997 (1997-02-07), pages 261-271
   D5: 'Immunological screening of drugs of abuse and gas chromatographic-mass spectrometric confirmation of opiates and cocaine in hair','SEGURA J ET AL','JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS,NL,ELSEVIER SCIENCE PUBLISHERS',",724/1/05-03-1999,9-21,

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

2. Claim 14 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

3.1 With respect to claim 1

Document D1, which is considered to represent the most relevant state of the art, discloses

a screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample (abstract) which includes the steps

of:
a) preparing a solid sample in a finely divided or powdered form (column 3, lines 18, 19);
b) selecting a liquid reagent providing constant concentration of hydroxyl groups (column 2, lines 53-61);
c) extracting cocaine and other similar substances contained in the sample and maintaining the sample completely immersed in said liquid reagent at a temperature ranging from 10°C to 250°C for a period of time ranging from few seconds to 48 hours (column 4, lines 14-17); and
d) analysing the liquid separated from the solid sample to determine the concentration of cocaine contained in said liquid with respect to the cut-off limit using a conventional screening kit for the determination of said substance in urine (column 2, lines 64-67) from which the subject-matter of claim 1 differs in that cocaine and other similar substances contained in the sample are transformed into benzoylecgonine and analysed.

The subject-matter of claim 1 is therefore novel (Article 33(2) PCT).

The problem to be solved by the present invention may therefore be regarded as to provide a screening process for quantitative determination of cocaine and other alkaloid wherein the analysis can effectively be performed by GC or GC/MS.

The solution to this problem proposed in claim 1 of the present application is considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
None of the documents cited gives an indication that the transformation of cocaine into benzoylecgonine permits the use of GC or GC/MS for the determination of cocaine and other alkaloids in a screening process. Prior screening techniques provide no transformation of cocaine into benzylecgonine and accordingly do not permit to use a GC or GC/MS for determination of such substances.

3.2 Claims 2-13 are dependent on claim 1 and as such also meet the requirements of the PCT with respect to novelty and inventive step.

INTERNATIONAL SEARCH REPORT

Inte[illegible]pplication No
PCT/IB 00/00966

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/94 G01N33/493

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 910 419 A (BROWN W CRAIG ET AL) 8 June 1999 (1999-06-08) abstract column 1, line 11 -column 5, line 28; tables 1,2 --- -/--	1,2,4, 6-8, 11-13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 2001	20/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Joyce, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SEGURA J ET AL: "Immunological screening of drugs of abuse and gas chromatographic-mass spectrometric confirmation of opiates and cocaine in hair" JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS,NL,ELSEVIER SCIENCE PUBLISHERS, vol. 724, no. 1, 5 March 1999 (1999-03-05), pages 9-21, XP004159001 ISSN: 0378-4347 page 10, column 2, paragraph 2 -page 11, column 2, paragraph 3 ---	1,2,5, 11,12,15
A	WO 93 03368 A (PSYCHEMEDICS CORP) 18 February 1993 (1993-02-18) page 8, line 16 - line 27 page 10, line 34 -page 11, line 8 page 18, line 8 - line 34 page 22, line 6 - line 18 page 25, line 5 - line 33 ---	1,2,4,6, 15
A	TAGLIARO F ET AL: "Hair analysis, a novel tool in forensic and biomedical sciences: new chromatographic and electrophoretic/electrokinetic analytical strategies" JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL SCIENCES & APPLICATIONS,NL,ELSEVIER SCIENCE PUBLISHERS, vol. 689, no. 1, 7 February 1997 (1997-02-07), pages 261-271, XP004054195 ISSN: 0378-4347 page 264, column 2, line 1, paragraph 2 -page 264, column 2, line 10, paragraph 2 -----	15

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte[illegible]pplication No
PCT/IB 00/00966

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5910419	A	08-06-1999	NONE		
WO 9303368	A	18-02-1993	AT	142020 T	15-09-1996
			CA	2092917 A	31-01-1993
			DE	69213207 D	02-10-1996
			EP	0555440 A	18-08-1993
			JP	6503424 T	14-04-1994
			US	5466579 A	14-11-1995
			US	6022693 A	08-02-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number
WO 01/06251 A2

(51) International Patent Classification[7]: G01N 33/48

(21) International Application Number: PCT/IB00/00966

(22) International Filing Date: 14 July 2000 (14.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
VR99A000059 20 July 1999 (20.07.1999) IT

(71) Applicant *(for all designated States except US)*: **AZIENDA PROVINCIALE PER I SERVIZI SANITARI** [IT/IT]; Via De Gasperi, 79, I-38100 Trento (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **VITALONE, Vincenzo** [IT/IT]; 30, Via Formigheta, I-38040 Martignano (IT). **LOTTI, Andrea** [IT/IT]; Via Morielle, 27, I-38063 Avio (IT). **GOTTARDI, Massimo** [IT/IT]; Via Bonfanti, 18, I-38034 Cembra (IT).

(74) Agent: **MAROSCIA, Antonio**; Maroscia & Associati S.r.l., Corso Palladio, 42, I-36100 Vicenza (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/06251 A2

(54) Title: PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS

(57) Abstract: A screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample and a reagent for use in the process. The process includes the steps of providing a solid sample in a finely divided or powdered form; completely immersing the sample into a liquid reagent providing a constant concentration of hydroxyl groups; maintaining the sample immersed in the liquid at a temperature in a range from 10 to 250°C for a period of time in a range from a few seconds to 48 hours; and analyzing the liquid separated from the solid with a conventional kit for the determination of the said substances in urine. The reagent is a liquid that provides a constant concentration of hydroxyl groups in the range of from 0.04 to 0.33 M and comprises 0.2 M $(NH_4)_2HPO_4$ with the addition of 5 ml of 25 % NH_4OH for each liter thereof.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number
WO 01/06251 A3

(51) International Patent Classification[7]: G01N 33/94, 33/493

(21) International Application Number: PCT/IB00/00966

(22) International Filing Date: 14 July 2000 (14.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
VR99A000059 20 July 1999 (20.07.1999) IT

(71) Applicant *(for all designated States except US)*: **AZIENDA PROVINCIALE PER I SERVIZI SANITARI** [IT/IT]; Via De Gasperi, 79, I-38100 Trento (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **VITALONE, Vincenzo** [IT/IT]; 30, Via Formigheta, I-38040 Martignano (IT). **LOTTI, Andrea** [IT/IT]; Via Morielle, 27, I-38063 Avio (IT). **GOTTARDI, Massimo** [IT/IT]; Via Bonfanti, 18, I-38034 Cembra (IT).

(74) Agent: **MAROSCIA, Antonio**; Maroscia & Associati S.r.l., Corso Palladio, 42, I-36100 Vicenza (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

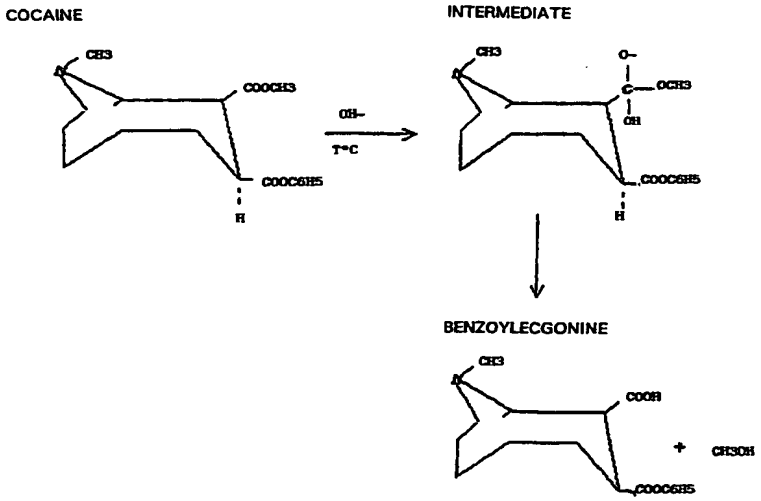
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
9 August 2001

[Continued on next page]

(54) Title: PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS



(57) Abstract: A screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample and a reagent for use in the process. The process includes the steps of providing a solid sample in a finely divided or powdered form; completely immersing the sample into a liquid reagent providing a constant concentration of hydroxyl groups; maintaining the sample immersed in the liquid at a temperature in a range from 10 to 250°C for a period of time in a range from a few seconds to 48 hours; and analyzing the liquid separated from the solid with a conventional kit for the determination of the said substances in urine. The reagent is a liquid that provides a constant concentration of hydroxyl groups in the range of from 0.04 to 0.33 M and comprises 0.2 M $(NH_4)_2HPO_4$ with the addition of 5 ml of 25 % NH_4OH for each liter thereof.

WO 01/06251 A3

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS.

This invention relates to a process for the quantitative determination of cocaine and other alkaloids, such as morphine and methadone, in a solid sample, e.g., a sample of hair, by using a screening type approach.

The invention also relates to a reagent for use in such process and new diagnostic kits including such reagent among their components.

By "screening type approach" is meant a kind of analysis permitting to analyze in a relatively short time span a relatively large number of samples in a cheap, efficacious and standardized manner. This kind of analysis permits to exclude the negative samples by immediately identifying the samples that do not contain the substance or the entire substance class or those in which said substances are present at a level lower than the threshold or cut-off value.

The threshold or cut-off is a practical limit selected to establish if the sample analyzed is positive or negative. The threshold value differs from the limit of detection of the method, that is, the lowest concentration of an analyte that can be determined. In fact, the cut-off value is normally set at a concentration higher than the limit of detection in order to obviate the imprecision of the analysis at values close to the limit of detection.

Cut-off values are conventionally established and take into account a multiplicity of factors, such as the capability of using commercially available reagents, the pharmacokinetic properties of the substances and the need to avoid false negatives (see: P.Zuccaro, S.Pichini, I.Altieri and R.Pacifici: Proposta di linee guida per l'analisi delle sostanze d'abuso nei liquidi biologici

in RAPPORTI ISTISAN 96/29 edited by ISTITUTO SUPERIORE DI SANITA').

The substances that the metabolism of the human body accumulates in the hair are numerous. Usually, it is required to detect the presence of alkaloids and other substances of abuse, such as, morphine, methadone and/or cocaine. By the method of this invention further substances can also be detected, such as, those of the following non-limitative list: 6-O- mono-acetyl-morphine, bi-acetyl-morphine, codeine, papaverine, nalorphine, nicotine, cotinine, caffeine, noscarpine, mepivacaine, trimetropin, buprenorphine, pentazocyne, methadone metabolyte, benzoylecgonine, amphetamine, metamphetamine, methylenedioxyamphetamine, methylenedioxymetamphetamine, methylenedioxyethylamphetamine, benzodiazepines, barbiturates.

Description of the state of the art

Several techniques are known for the determination of the analytes of interest, such as those mentioned above. Specifically, for the analysis of cocaine that is present in hair, gas chromatography (GC) combined with Mass Spectrometry (MS) hereinafter referred to as GC/MS and Radio Immune Assay technique (R.I.A.) are known.

GC or liquid chromatography (LC) combined with MS are methods of resolution, purification or separation and identification of components of complex mixtures of organic or inorganic substances having even strictly similar chemical properties. The separation of substances dissolved in a liquid or fixed on a finely divided or porous solid substance is based on percolation, respectively elution trough them of an eluent gas, respectively liquid.

When the substances to separate/detect can be made gaseous and as eluent a

gas is used, GC applies. The latter is a separation method based on the distribution between a solid or liquid stationary phase and a mobile phase made of the gases or vapors to separate, which are carried by a stream of an inert gas.

The analytical results are reported in a graph named chromatogram, in which the quantities of the single components present in the mixture and transferred to the eluent gas/liquid are reported versus time. The graph has peaks whose highness is a direct function of the quantity of the specific substance.

Chromatographic methods that can be used for confirmatory analyses are GC and LC, the latter being often referred to as High Performance Liquid Chromatography (HPLC). The most commonly employed detectors for GC are electron scatting detectors and phosphor nitrogen detectors.

As said above, GC/MS employs a gas chromatograph coupled to a mass spectrograph. By so doing, the separation capability of GC combines with the specificity proper of MS. Therefore, GC/MS represents the method of choice for confirmatory analyses of the above named substances as well as their metabolites (see again "Proposta di linee guida per l'analisi delle sostanze d'abuso nei liquidi biologici" by ISTITUTO SUPERIORE DI SANITA')

In the conventional acid hydrolysis, however, cocaine is extracted by hydrochloric acid as it is, i.e. without undergoing transformation into its metabolite benzoylecgonine. Since the amounts of cocaine extracted in this way are very little, it is not possible to determine cocaine by the usual screening methods.

Radio Immune Assay (RIA) is based on radio-immunological tests using known amounts of antibodies and of analyte labeled with a radioisotope (generally

I^{125}). During incubation the labeled analyte and that possibly present in the sample compete for the antibody sites. After precipitation of antigen-antibody complexes and centrifugation the supernatant or the precipitate are transferred to a gamma Geiger counter that measures the radioactivity level. RIA kits are extremely sensitive and allow identification of 1-5 ng of substance per ml. Adoption of automatic instruments for pipetting and counting allows the contemporaneous analysis of numerous samples with response times of 1 to 5 hours. On the other hand, the use of radioactive isotopes requires adequate safety measures. Furthermore, the relatively short half-life of the radioactive isotopes imposes a careful handling of reagents.

RIA does not lend itself to a widespread use for the quick determination of the substances in question in view, first of all, of the high cost of reagents, further increased by liability of the antisera. Secondly, in view of the criticality of the analysis due to the need of operating with radioactive materials that are dangerous for the analysts and also in view of the required times which are relatively long. RIA also requires the availability of rooms properly equipped and shielded, as well as particular care in handling waste materials and in their disposal.

Therefore, RIA is scarcely employed in this sort of analyses. The prior art shows therefore the impracticality of determining cocaine present in the hair by the so-called screening type approach, as described above, i.e., by very quick and cheap techniques as are used for the determination of the same substances in urine. This is mainly due to the fact that in the conventional screening-type approach what is analyzed is not cocaine, but its metabolite benzoylecgonine. The fact that this substance does not exist in nature by itself, but only as the metabolyte of cocaine and the fact that the reaction of transformation of cocaine into benzoylecgonine, i.e. the transformation of the ester group into an hydroxyl group, is irreversible in an alkaline environment

render the determination of benzoylecgonine a necessary and sufficient condition for the detection of cocaine.

Benzoylecgonine is found in the hair in a ratio of 1 to 4 or even 1 to 10 and more with respect to cocaine. Therefore, when the amount of cocaine is very low or close to the cut-off values (0.2-0.1 ng cocaine per mg hair) screening cannot be performed because of the low amount of this metabolyte (0.05-0.025 ng benzoylecgonine per mg hair), such amount being undetectable by the screening apparatuses available in the laboratories.

Summary of the invention

The main object of this invention is to overcome the above mentioned drawbacks, i.e. the impossibility to dose the cocaine present in a solid sample by the conventional screening-type approach.

In accordance with the invention, this object is achieved by a screening-type procedure for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which includes the following steps:

- providing a solid sample in a finely divided and/or powdered form;
- completely immersing the sample into a liquid reagent providing a concentration of hydroxyl groups in the range of from 0.0001 to 5 M;
- maintaining the sample immersed in the liquid at a temperature of 10 to 250°C for a period of time from a few seconds to 48 hours; and
- analyzing the liquid separated from the solid with a conventional kit for the determination of the said substances in urine.

Preferred aspects of the invention include using hair as the solid material forming the solid sample; using a buffer as the reagent which provides hydroxyl groups, more preferably an ammonia buffer, most preferably a buffer

that is hereinafter referred to as VMA; and heating the sample immersed in the liquid at a temperature of about 100 to 150°C for about one hour.

In accordance with another aspect of the invention a process is provided which includes the additional steps of:

- arranging samples by increasing concentrations of the substances of interest; and
- performing confirmation analyses with known techniques, such as, GC or GC/MS.

In one embodiment of the invention, the process may provide the following steps:

- providing a sample made of about 50 to 300 mg of finely divided and/or powdered material;
- adding in the test tube containing the said sample a suitable liquid reagent until the sample is completely immersed, said reagent being capable of performing extraction and transformation of cocaine into benzoylecgonine and at the same time of extracting other similar substances which are present in the sample
- if necessary, agitating the test tube to facilitate immersion of the sample;
- heating the contents of the test tube to a temperature T1 for a time interval t1 by keeping the test tube immersed in a thermostated bath or by placing it in an oven;
- cooling the test tube to room temperature;
- taking the liquid and transferring it into a test tube suitable for a screening type instrument;
- performing the screening by using a kit of reagents for the determination of the said substances in urine;
- reading the data resulting from the first level instrumentation to verify the concentration values with respect to the cut-off limit; and

- contemporaneously determining the amount(s) of substance(s) present.

In accordance with another aspect of the invention a reagent is provided for use in the above mentioned process which in the most preferred embodiment has the following formula:

$$VMA = 0.2\ M\ (NH_4)_2HPO_4 + 5ml/L\ 25\%\ NH_4OH$$

In the above formula $(NH_4)_2HPO_4$ is dibasic ammonium phosphate and NH_4OH is ammonium hydrate. In fact, 5ml/L NH_4OH give a 0.07 M concentration of hydroxyl groups, which is comprised in the range giving 100% conversion.

In addition to the main advantage of the invention as outlined above, another advantage is that the confirmation analyses (e.g., GC/MS) need to be performed only on those samples that have been determined to be positive at the initial screening whereas, when conventional techniques are used, all samples must be analyzed since there is no kit available for determination of cocaine, but only for determination of benzoylecgonine.

In average, the analysis of 25 samples requires two hours for the preparation of the samples plus one week for the confirmation analyses of all (both positive and negative) samples.

On the other hand, by applying the present invention, an analyst will require for the same 25 samples two hours for samples preparation plus 30 minutes for the screening-type analysis plus the time necessary for performing the confirmation analyses. However, the latter need to be performed only on those samples which have been determined to be positive.

Since, in the average, positive samples are between 0 and 20% and since

most of the time required is spent in the confirmation analyses, the procedure according to the present invention allows saving of substantial time which is estimated at about 70 to 90% of the time required for the analysis of the 25 samples taken into consideration.

Another advantage according to this invention is that the possible dragging of the active substances from one sample to another in the confirmation analyses is eliminated or minimized because it is possible to arrange samples in order of increasing concentration of the substances of interest, therefore proceeding to confirmation analyses starting from those samples having the lowest concentration and then with those having higher and higher concentrations.

In this way, it is completely eliminated the possibility that a sample with high concentration of, e.g. cocaine, leaves a trace of it in the instrument used for the analysis and has an impact on the determination of the same substance in the following sample, perhaps having a concentration just below the cut-off.

Still another advantage of this invention is that it allows to search and determine at the same time and in the same sample not only cocaine but also other substances, such as, morphine by applying the same cut-off limit or else methadone by modifying the limit of cut-off.

One further advantage is that the procedure according to this invention offers a higher guarantee to the analyst because positive outcome is confirmed by two different analytical methods.

In a further aspect of the invention a diagnostic kit is provided including the reagent described above as one of its components.

Still further additional advantages will appear from the reading of the following detailed description and the following non-limitative examples.

Brief Description of the Drawings

Figure 1 is a chromatogram obtained from an apparatus performing gas chromatography on the sample of Table 2;

Figure 2 is the graph obtained by Mass Spectrometry on the first sample after acid hydrolysis and shows presence of cocaine;

Figure 3 is the Mass Spectrum of the same first sample after treatment according to the present invention and shows presence of benzoylecgonine;

Figure 4 is the Mass Spectrum of the same first sample after treatment according to the present invention and shows presence of morphine;

Figure 5 is a chromatogram similar to that of Figure 1 and refers to the first sample treated according to this invention as shown in Table 3;

Figure 6 is similar to the MS of Figure 3 showing presence of benzoylecgonine;

Figure 7 is similar to the MS of Figure 3 showing presence of morphine;

Figure 8 is similar to Figure 1 but relates to a second sample;

Figure 9 is similar to Figure 5 but relates to a second sample;

Figures 10 and 11 are respectively similar to Figures 8 and 9 but relate to a

third sample;

Figures 12 and 13 are respectively similar to Figures 8 and 9 but relate to a fourth sample;

Figure 14 is a diagram showing transformation of cocaine into its metabolite benzoylecgonine;

Figure 15 is a graph showing concentration of cocaine versus concentration of OH^- when the reaction of transformation of cocaine into benzoylecgonine occurs at 100° C for 1 hour; and

Figure 16 is similar to Figure 15 and shows the case in which the reaction occurs at 150°C for 1 hour.

Detailed description of invention embodiments

The process of this invention substantially is a process for the quantitative extraction and transformation of cocaine into benzoylecgonine and for the extraction of similar alkaloids, in particular toxic substances of abuse and/or drugs, which are present in a sample, prepared starting from a solid material.

In the following detailed example, the sample is obtained starting from finely divided hair.

The procedure allows, in particular, the dosage of cocaine by a screening type technique, at the same time allowing the dosage of possible other toxic substances and the like present in the hair, by using kits of reagents available in commerce and intended for the analysis in urine.

The following steps are carried out:

- crush hair in fragments of two-three mm length;
- weigh the fragments to form a sample having a weight of 50 to 300 mg;
- wash the sample with methanol in a closed test tube at room temperature to eliminate possible external substances that might interfere with the results, such as, for example, traces of drugs external to the hair that have deposited on it because of its presence in the air (by so doing, it is possible to distinguish if the hair was that of a handler or that of the consumer of the drug);
-Repeat the step of washing with methanol, if necessary;
- wash the sample with ethanol in a closed test tube to eliminate traces of methanol or water;
- dry in an oven at about 45°C under flow of inert gas, such as, nitrogen;
- add into the test tube 0.5 to 2 ml of the reagent as defined above and shake if necessary;
- heat the test tube to 100°C and maintain at this temperature for 1 hour, e.g. by means of a thermostatic bath or an oven;
- cool the test tube to room temperature, e.g., by immersing the tube into cold water or simply leaving it at room temperature for a suitable time span;
- take the liquid and pour into a test tube of the kind used for urine examination (if necessary, centrifuge the test tube to eliminate turbidity);
- insert the tube in a "first-level screening apparatus" for the quantitative determination of the substances sought for (benzoylecgonine, morphine, methadone, etc);
- adjust settings of the first-level apparatus in a way suitable for small amounts (this may be necessary if the apparatus is alternatively used for determination in hair or urine);
- perform screening –type analyses using the reagents provided with the kit normally employed for the examination of urine;
- read data resulting from the first-level analyses and establish positivity or negativity with reference to the cut-off value.

The above procedure may then be complemented with the following additional steps when confirmation analyses are required:

- arrange the samples in the order of increasing concentration of the sought for substances, determined as above described;
- perform confirmation analyses by analyzing the samples taken in the order of the arrangement (In this way the above mentioned problems of dragging traces of drugs from one sample to the other are overcome).

The invention process provides for the use of a reagent (hereinafter referred to as VMA) which is a buffer solution. The buffer serves for transforming the cocaine present in the hair into its metabolite benzoylecgonine, as shown in the scheme of Figure 14. Cocaine, in the presence of hydroxyl groups and at a suitable temperature first is transformed into an intermediate product and then into benzoylecgonine plus methanol.

Buffer solutions are obtained by reacting a salt with its weak base. These solutions have a stable pH; therefore the VMA reagent is able to produce hydroxyl groups in a steady way. The use of solutions in which the production of hydroxyl groups is not regular creates problems when the cocaine concentration is close to the cut-off limit.

As said above, the composition of the buffer for use in the process is preferably the following:

$$VMA = 0.2\ M\ (NH_4)_2HPO_4 + 5ml/L\ 25\%\ NH_4OH$$

Alternatively, VMA may be replaced by solution in which the component being the source of hydroxyl groups is selected among the following non-limitative list of substances: aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium

hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide, potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate, tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide.

As solvent, any of the following can be used in alternative:
ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

Figure 15 shows a graph in which the percentage of cocaine transformed into benzoylecgonine is reported versus the concentration of OH^- when the reaction is carried out at the temperature of 100°C for 1 hour. From the graph it appears evident that the transformation is maintained at high levels (at least 70%) for a range of OH^- concentrations of from 0.03 to 0.5 M.

From the graph of Figure 16, which shows hydroxyl concentrations after, respectively, 0, 15, 30 and 60 minutes in a reaction carried out at 150°C; it appears evident that the percentage of transformed cocaine is high only around the abscissa point of 15 minutes.

From all of the above and numerous other experiments the optimal values for the reaction temperature and time result to be, respectively, 100°C and 1 hour.

The examples reported below show the quantitative determination of cocaine in hair performed both with known techniques and with the process of the invention. Analyses have been carried out using the following instruments: for the screening analyses, the ROCHE instrumentation named "COBAS MIRA PLUS" which uses reagents provided by the same company and named "ABUSCREEN ON LINE"; for GC/MS the instrument provided by the company VARIAN which is named "SATURN GC/MS", model 4D.

EXAMPLES

Before proceeding to the various screening analyses the apparatus has been controlled with a sample positive to both cocaine and morphine in order to verify feasibility of the methodology.

As can be seen from Table 1 the sample resulted positive to both cocaine and morphine:

TABLE 1

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.16 ng/ml	POSITIVE
COCAINE	0.15 ng/ml	POSITIVE

The concentration values detected by the apparatus resulted to be 0.16 for morphine and 0.15 for cocaine, in line with the expected values for both substances present in the control sample.

EXAMPLE 1

In this example a sample has been analyzed which resulted, in the end, to be positive to both cocaine and morphine.

The sample was subjected to conventional analysis with acid hydrolysis and then subjected to screening analysis which gave the following results:

TABLE 2

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.13 ng/ml	POSITIVE
COCAINE	0.01 ng/ml	NEGATIVE

As can be seen, acid hydrolysis was able to extract an amount of morphine higher than the limit, but the amount of cocaine resulted to be too little to allow detection by the screening apparatus. The reason is that cocaine was extracted as such and not transformed into its metabolite benzoylecgonine, which latter is just what present screening methods detect.

The same sample was then subjected to confirmatory analysis by GC/MS with the results reported in Figures 1 to 4. Specifically, from Figure 1 it can be seen that the chromatogram of the first sample shows a high peak at the position of cocaine and a very small peak at the position of benzoylecgonine. The same Figure 1 also shows confirmation of the presence of morphine.

Additional confirmations of the presence of both cocaine and morphine result from the graphs of Figures 2, 3 and 4.

The sample of Example 1 was then subjected to the procedure of this invention to give the results shown in Table 3.

TABLE 3

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.23 ng/ml	POSITIVE
COCAINE	0.37 ng/ml	POSITIVE

It is immediately clear that the values detected for both morphine and cocaine are higher than the respective limits, therefore the sample is positive for both.

The high value detected for cocaine is to be ascribed to the benzoylecgonine that has been produced during the transformation reaction.

The same sample of Example 1 treated with the process of this invention has been subjected to GC/MS confirmation analyses with the results reported in Figures 5 to 7. As can be seen from Figure 5, the cocaine peak practically cannot be seen anymore because cocaine has been completely transformed in benzoylecgonine, the peak of which is well apparent in the same Figure 5.

EXAMPLE 2

Again in this example a sample which, in the end, resulted to be positive to both cocaine and morphine has been analyzed first by the conventional analytical method of acid hydrolysis and screening with the results reported in Table 4.

TABLE 4

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.32 ng/ml	POSITIVE
COCAINE	0.03 ng/ml	NEGATIVE

Like in the preceding Example, acid hydrolysis was able to extract an amount of morphine higher than the limit, but the quantity of cocaine extracted was too little to allow detection by the apparatus.

The sample was then subjected to GC/MS confirmatory analyses to give the results reported in Figure 8 where it can be seen that the chromatogram shows a high peak at the position of cocaine and a negligible peak at the position of benzoylecgonine.

The sample of Example 2 was then subjected to the invention process and to screening analyses with the results reported in Table 5.

TABLE 5

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.28 ng/ml	POSITIVE
COCAINE	0.99 ng/ml	POSITIVE

Both the cocaine and the morphine values are higher than the respective limits, therefore the sample is positive to both.

The sample of this Example, treated according to the invention process, was also subjected to GC/MS confirmation analyses with the results reported in Figure 9 where it can be seen that the cocaine peak is not practically present anymore whereas the benzoylecgonine peak is high.

EXAMPLE 3

In this Example a sample has been analyzed which, at the end, resulted to be positive to cocaine only and not to morphine.

This sample was first subjected to acid hydrolysis and screening by the conventional methods to give the results reported in Table 6.

TABLE 6

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.01 ng/ml	NEGATIVE

By acid hydrolysis a quantity of morphine lower than the limit was extracted. The quantity of cocaine was also too little to allow the equipment to detect it. By using the methods known in the art, therefore, nothing can be said as to the positivity or negativity of the sample and it was necessary to make recourse to the GC/MS confirmation analyses that gave the results reported in Figure 10. In such Figure, the chromatogram of the sample of this Example shows a high peak at the position of cocaine and a negligible peak at the position of benzoylecgonine. This confirms once mor that acid hydrolysis extracted cocaine as such without any transformation.

The same sample of this Example has also been treated according to the invention process and subjected to screening analysis. Results are reported in Table 7.

TABLE 7

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.03 ng/ml	NEGATIVE
COCAINE	0.13 ng/ml	POSITIVE

It is immediately evident that while the value of morphine is lower than the limit, that of cocaine is slightly higher than the cut-off and therefore the sample is only positive to cocaine.

This same sample, after treatment with the invention process, has been subjected to GC/MS confirmation analyses with the results shown in Figure 11. In this Figure, the peak of cocaine has practically disappeared because the same was completely transformed into benzoylecgonine, the peak of which is, instead, very high and well apparent.

EXAMPLE 4

This fourth Example also analyzes a sample which, at the end, resulted positive to cocaine and not to morphine.

Analyses were carried out by applying the same scheme as in the preceding Examples.

Table 8 shows that upon analysis with acid hydrolysis the sample seems to be completely negative to both morphine and cocaine, whereas Figure 12 indicates the presence of cocaine in an amount higher than the limit.

TABLE 8

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.05 ng/ml	NEGATIVE

The same sample, treated with the invention process (VMA reagent) already on the first screening analysis shows a presence of cocaine higher than the limit, see Table 9.

TABLE 9

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.64 ng/ml	POSITIVE

As usual, this result was confirmed by GC/MS analysis as shown in Figure 13. The big peak of benzoylecgonine indicates that, before the treatment, the sample contained cocaine in a percentage higher than the cut-off.

The instant application is based upon Italian patent application VR99A000059, filed on 20 July 1999, the disclosure of which is hereby expressly incorporated by reference thereto, and the priority of which is hereby claimed.

CLAIMS

1. A screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which includes the steps of:
   - providing a solid sample in a finely divided or powdered form;
   - completely immersing the sample into a liquid reagent providing a constant concentration of hydroxyl groups;
   - maintaining the sample immersed in the liquid at a temperature in a range from 10 to 250°C for a period of time in a range from a few seconds to 48 hours; and
   - analyzing the liquid separated from the solid with a conventional kit for the determination of the said substances in urine.

2. Process according to claim 1, wherein the solid sample is a sample of hair.

3. Process according to claims 1 and 2, wherein said range of temperature is from 100 to 150°C.

4. Process according to claims 1 and 2, wherein said range of period of time is from 15 minutes to 24 hours.

5. Process according to any preceding claims, wherein said temperature is maintained at 100°C for 1 hour.

6. Process according to any of the preceding claims, wherein the concentration of hydroxyl groups is in the range of from 0.0001 to 5 M;

7. Process according to any of the preceding claims, wherein the concentration of hydroxyl groups is in the range of 0.03 to 0.5 M.

8. Process according to any preceding claims, wherein the concentration of hydroxyl groups is in the range of 0.04 to 0.33 M.

9. Process according to any of the preceding claims, wherein the liquid reagent is ammonia buffer.

10. Process according to claim 7, wherein the buffer is 0.2 M (NH4)2HPO4 with the addition of 5 ml of 25% NH4OH to each liter thereof.

11. Process according to any preceding claims, which further comprises the steps of:

- arranging the analyzed samples in the increasing order of concentration of drugs; and
- performing confirmation analyses with standard techniques of the samples taken in the said order.

12. Screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample, comprising the following steps:

- providing a sample made of about 50 to 300 mg of finely divided and/or powdered material;
- adding in the test tube containing the said sample a suitable liquid reagent until the sample is completely immersed, said reagent being capable of performing extraction and transformation of cocaine into benzoylecgonine and at the same time of extracting other similar substances which are present in the sample
- if necessary, agitating the test tube to facilitate immersion of the sample;
- heating the contents of the test tube to a temperature T1 for a time interval t1 by k eping the test tube immersed in a thermostated bath or by

placing it in an oven;

- cooling the test tube to room temperature;
- taking the liquid and transferring it into a test tube suitable for a screening type instrument;
- performing the screening by using a kit of reagents for the determination of the said substances in urine;
- reading the data resulting from the first level instrumentation to verify the concentration values with respect to the cut-off limit; and
- contemporaneously determining the amount(s) of substance(s) present.

13. Reagent for use in the process of claims 1, 9 or 10, which is a liquid that provides a constant concentration of hydroxyl groups in the range of from 0.04 to 0.33 M.

14. Reagent according to claim 12, which is 0.2 M $(NH_4)_2HPO_4$ with the addition of 5 ml of 25% NH_4OH for each liter thereof.

15. Reagent according to claim 11 or 12, wherein said solution comprises a solute selected among aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide,potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate, tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, dissolved in a solvent selected among ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium

carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

16. Use of the reagent according to claims 12 to 15 for the carrying out of the process of any of claims 1 to 10 and 11.

17. Diagnostic kit including the reagent of claim 12 as one of its components.

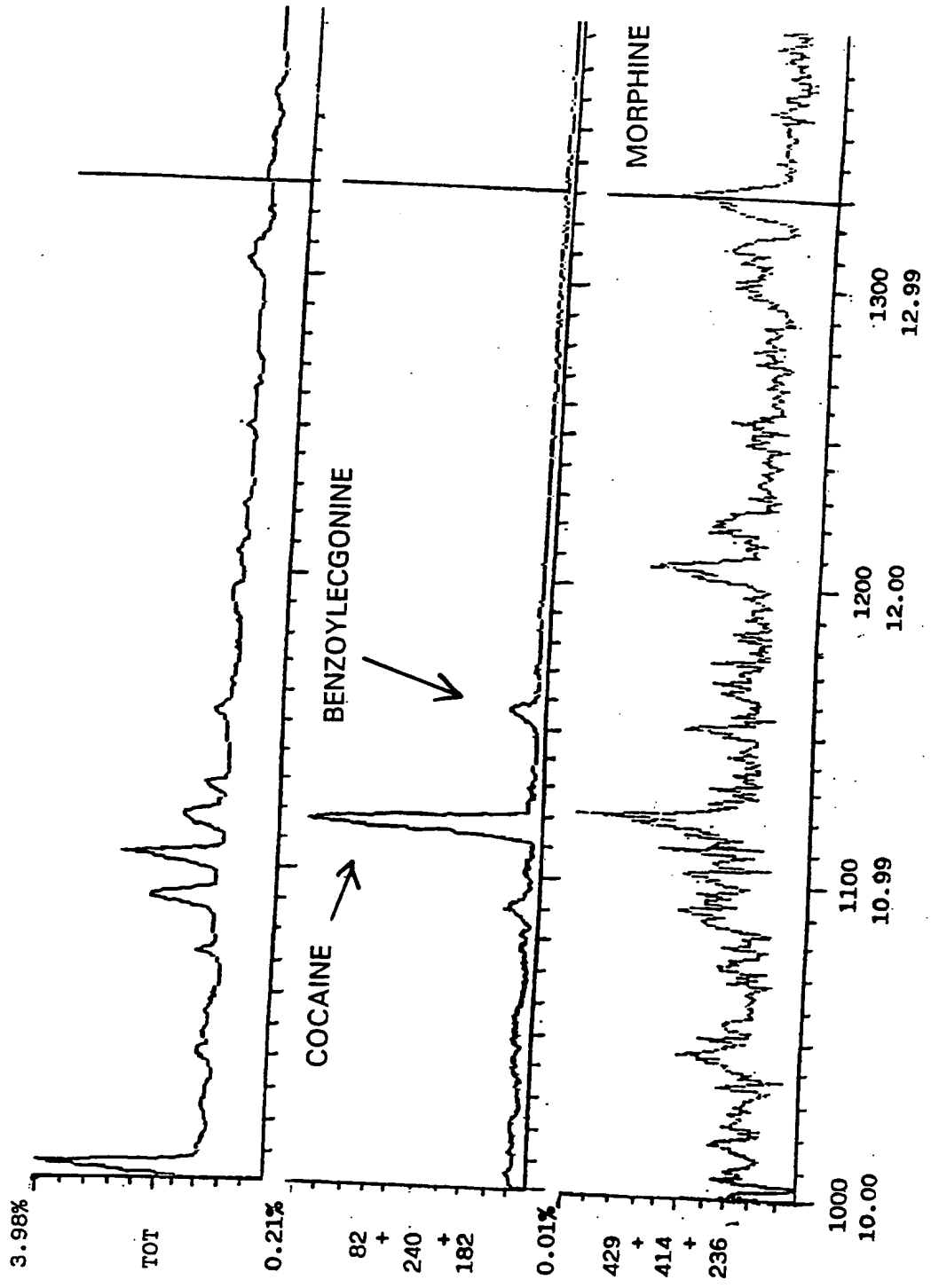

3.98%
TOT
0.21%
82
+
240
+
182
0.01%
429
+
414
+
236
BENZOYLECGONINE
COCAINE
MORPHINE
1000
10.00
1100
10.99
1200
12.00
1300
12.99

Fig. 1

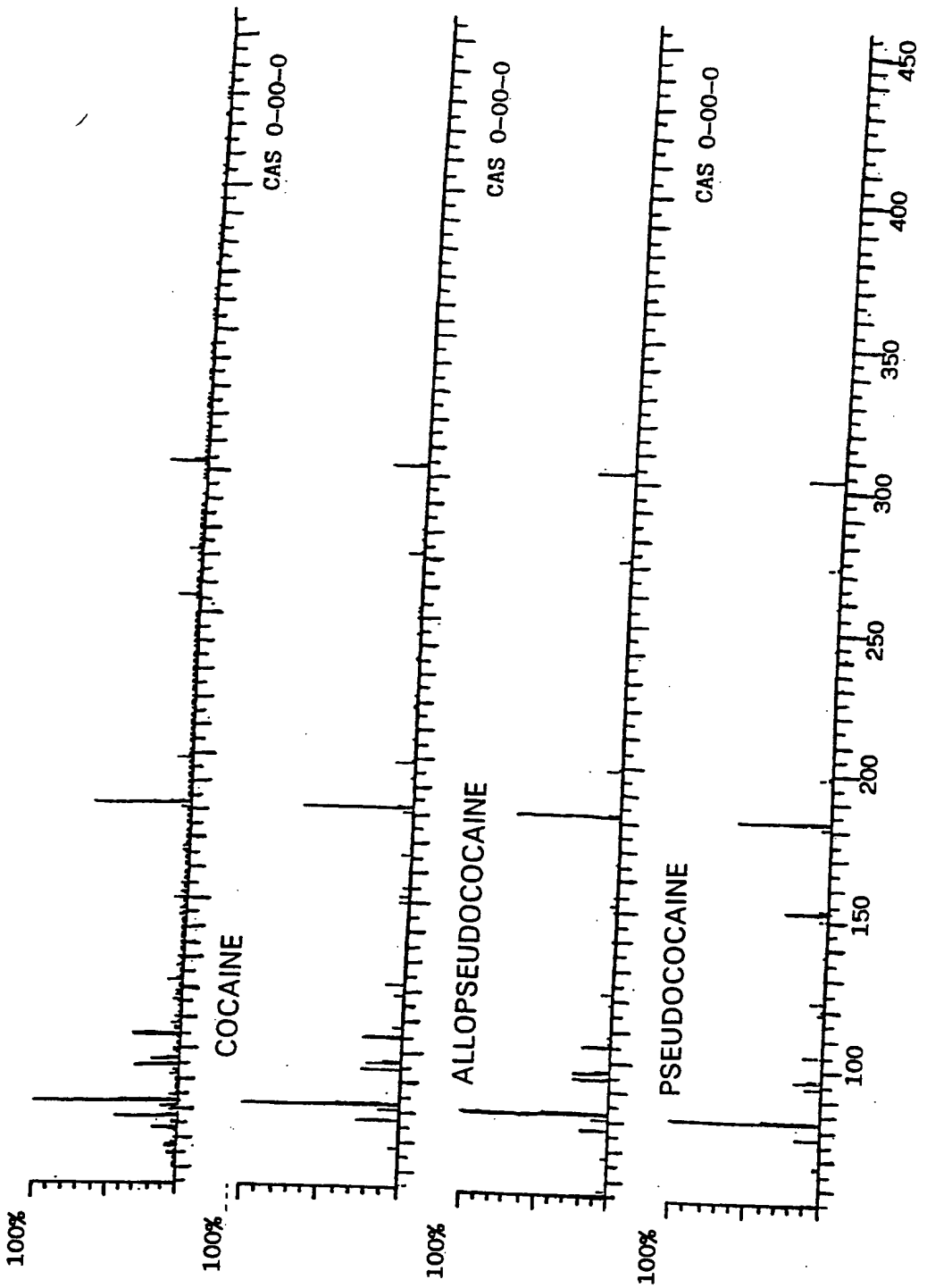

100%
COCAINE
CAS 0-00-0
100%
ALLOPSEUDOCOCAINE
CAS 0-00-0
100%
PSEUDOCOCAINE
CAS 0-00-0
100%
100
150
200
250
300
350
400
450

Fig. 2

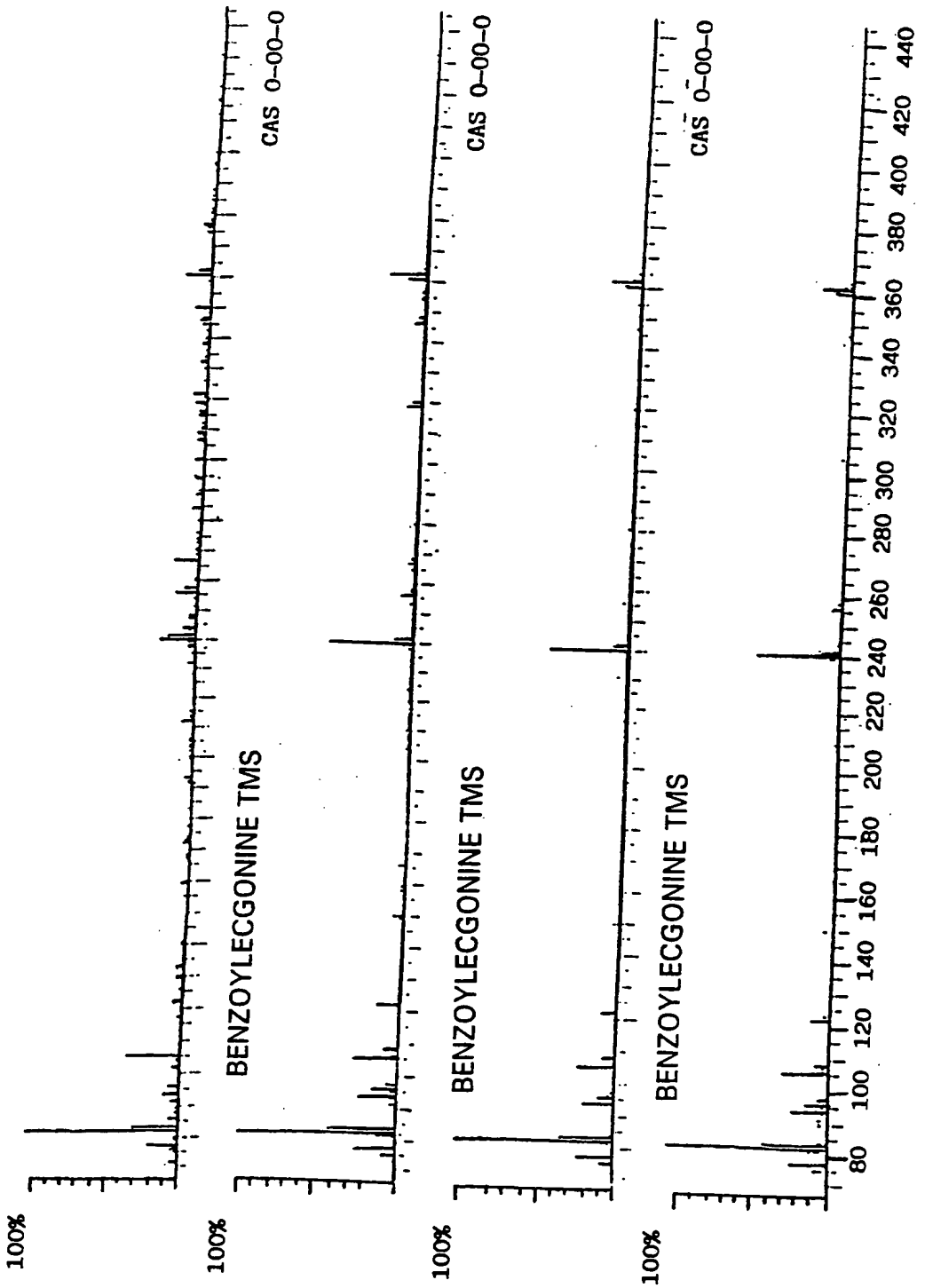

100%
BENZOYLECGONINE TMS
CAS 0-00-0
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440

Fig. 3

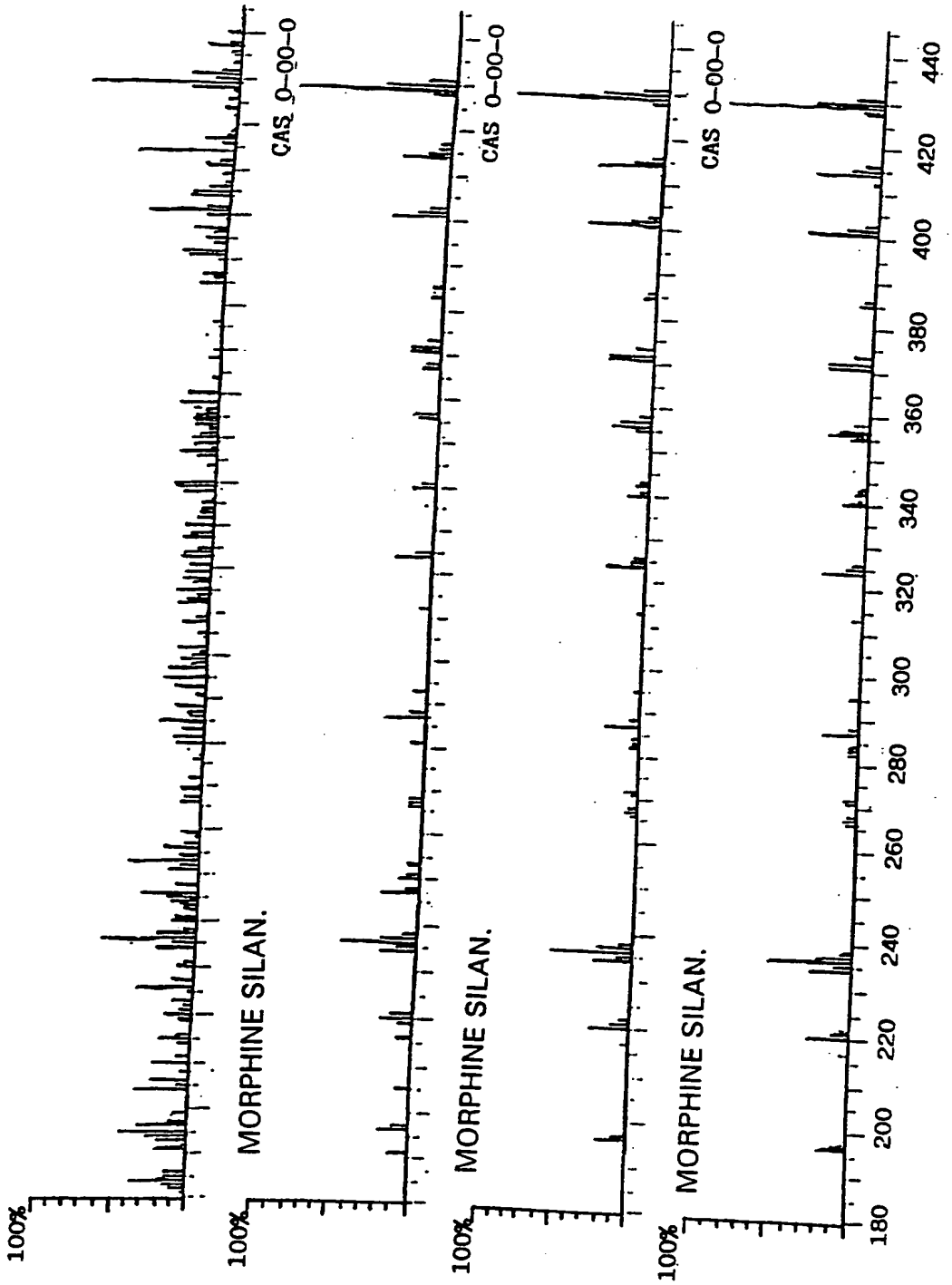

100%
MORPHINE SILAN.
CAS 0-00-0
100%
MORPHINE SILAN.
CAS 0-00-0
100%
MORPHINE SILAN.
CAS 0-00-0
100%
MORPHINE SILAN.
CAS 0-00-0
180
200
220
240
260
280
300
320
340
360
380
400
420
440

Fig. 4

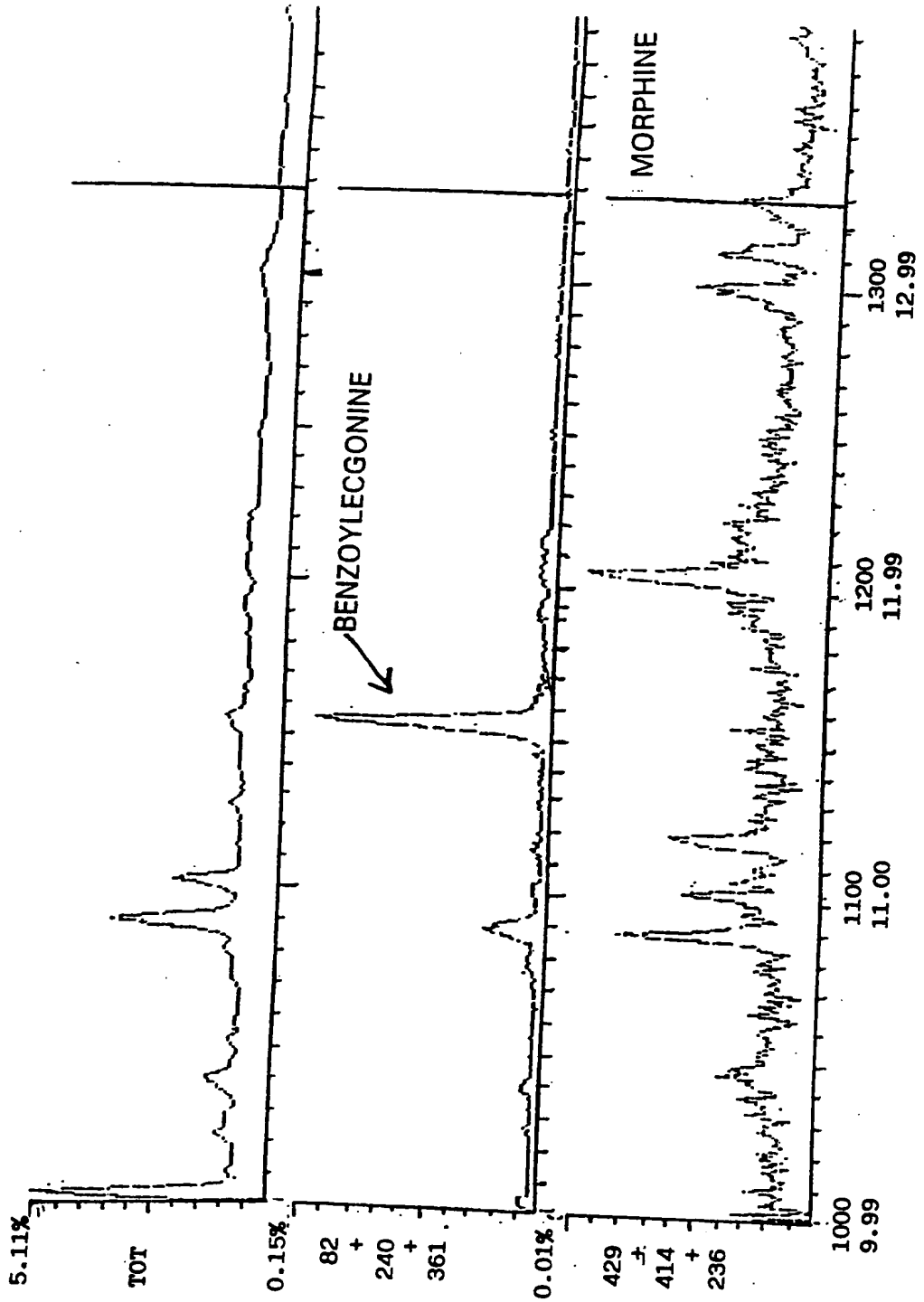

5.11%
TOT
0.15%
82
+
240
+
361
BENZOYLECGONINE
0.01%
429
+
414
+
236
MORPHINE
1000
9.99
1100
11.00
1200
11.99
1300
12.99

Fig. 5

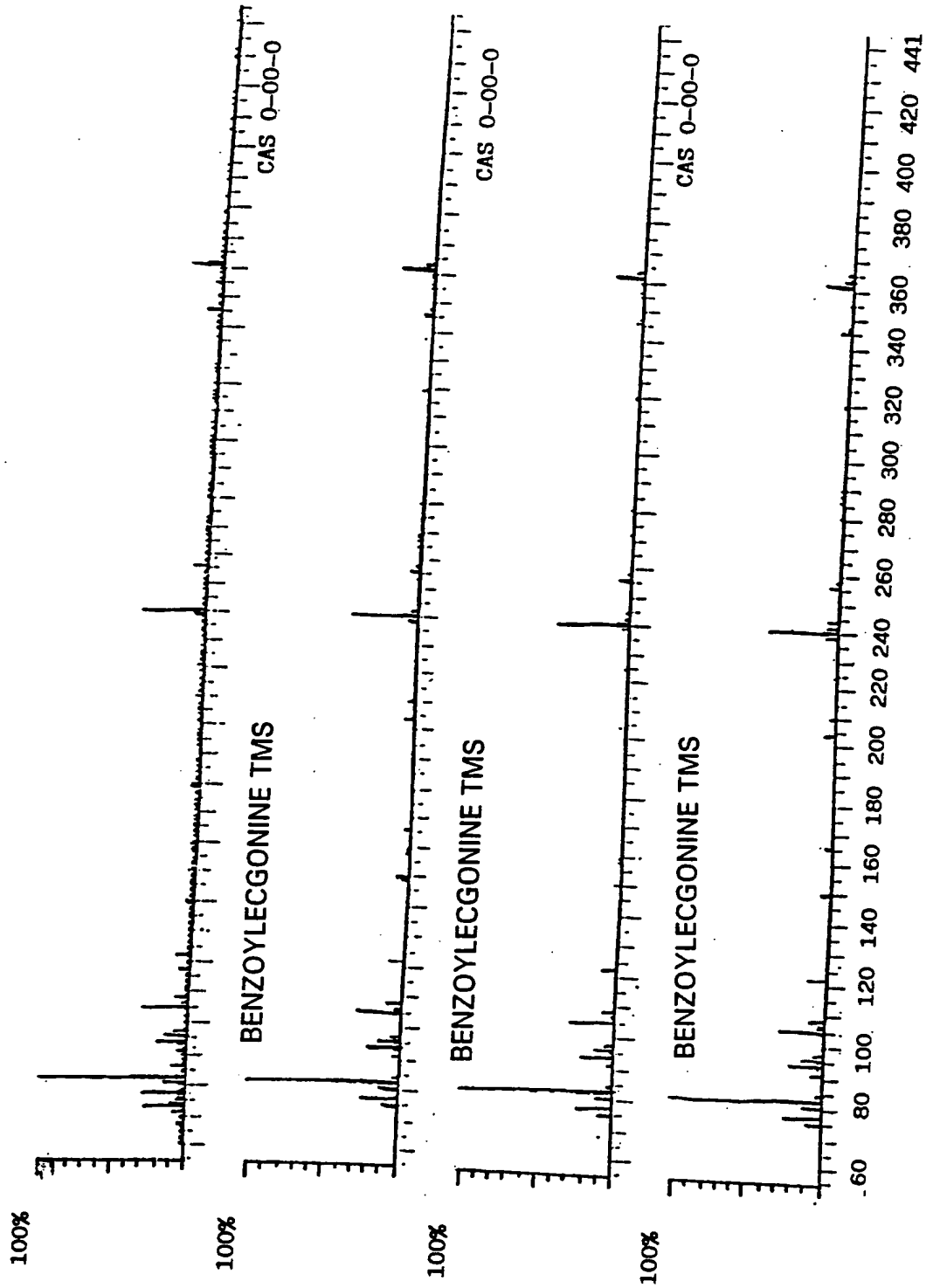

100%
BENZOYLECGONINE TMS
CAS 0-00-0
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 441

Fig. 6

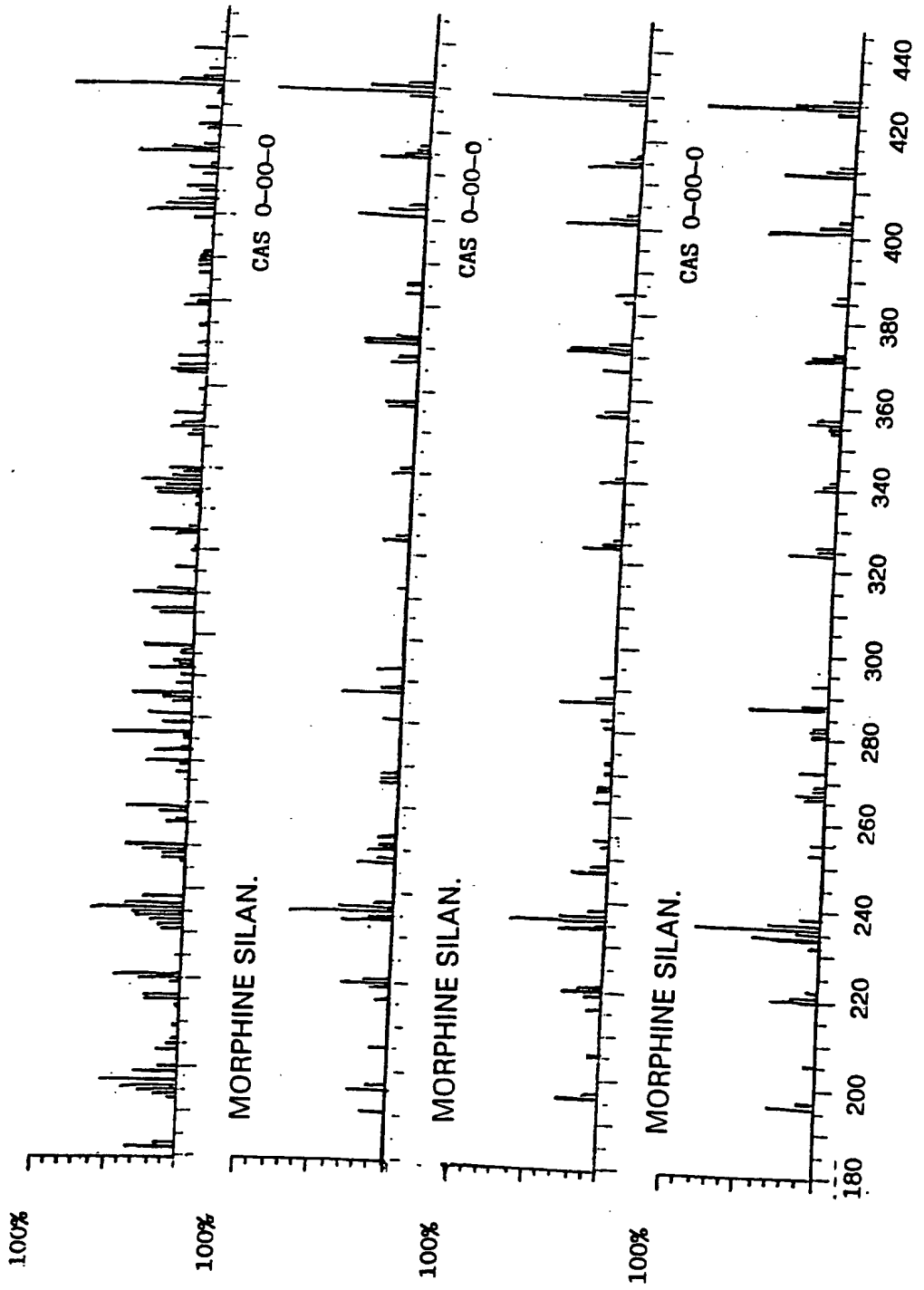

100%
MORPHINE SILAN.
CAS 0-00-0
100%
MORPHINE SILAN.
CAS 0-00-0
100%
MORPHINE SILAN.
CAS 0-00-0
100%
180
200
220
240
260
280
300
320
340
360
380
400
420
440

Fig. 7

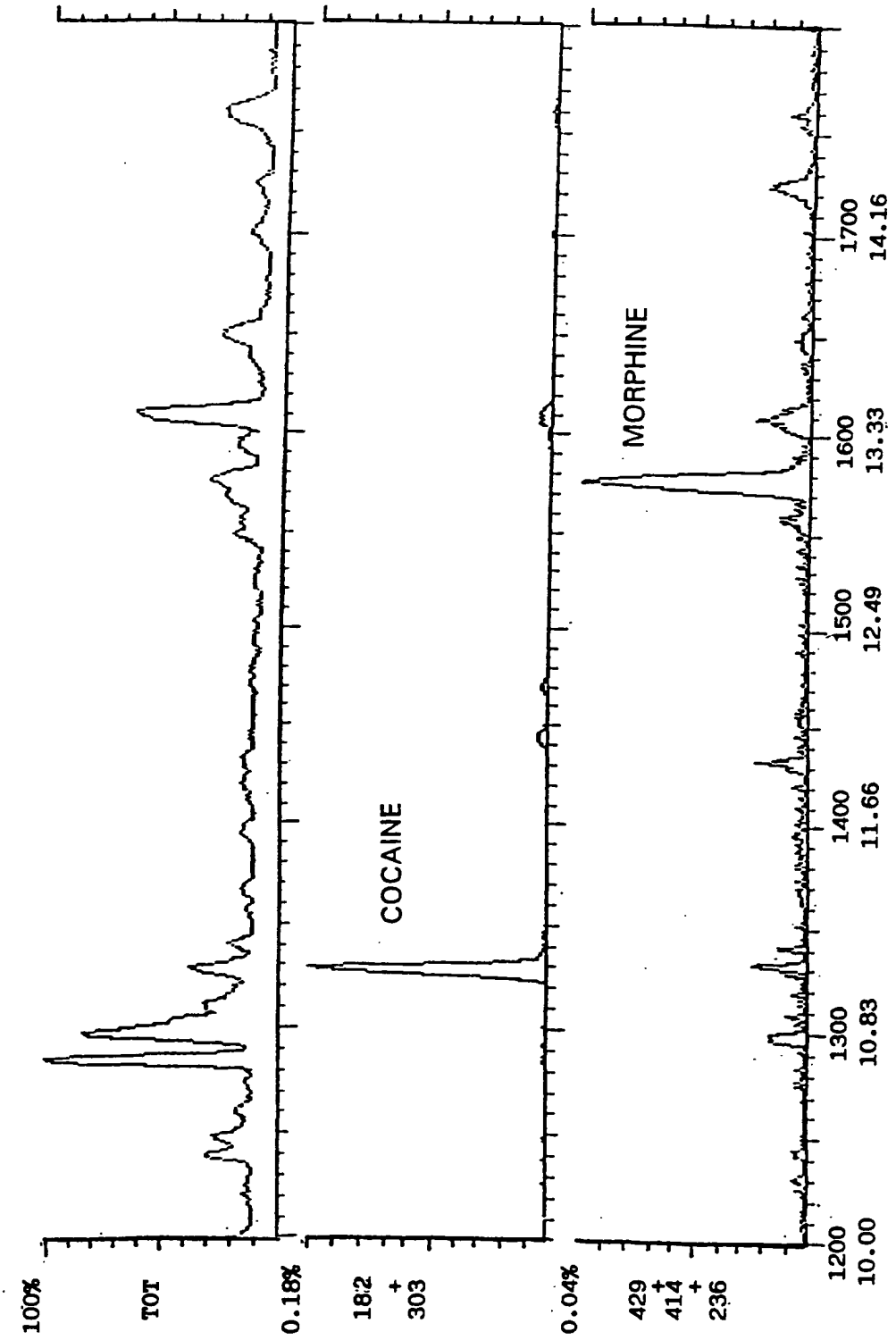

100%
TOT
0.18%
182
+
303
COCAINE
0.04%
429
+
414
+
236
MORPHINE
1200
10.00
1300
10.83
1400
11.66
1500
12.49
1600
13.33
1700
14.16

Fig. 8

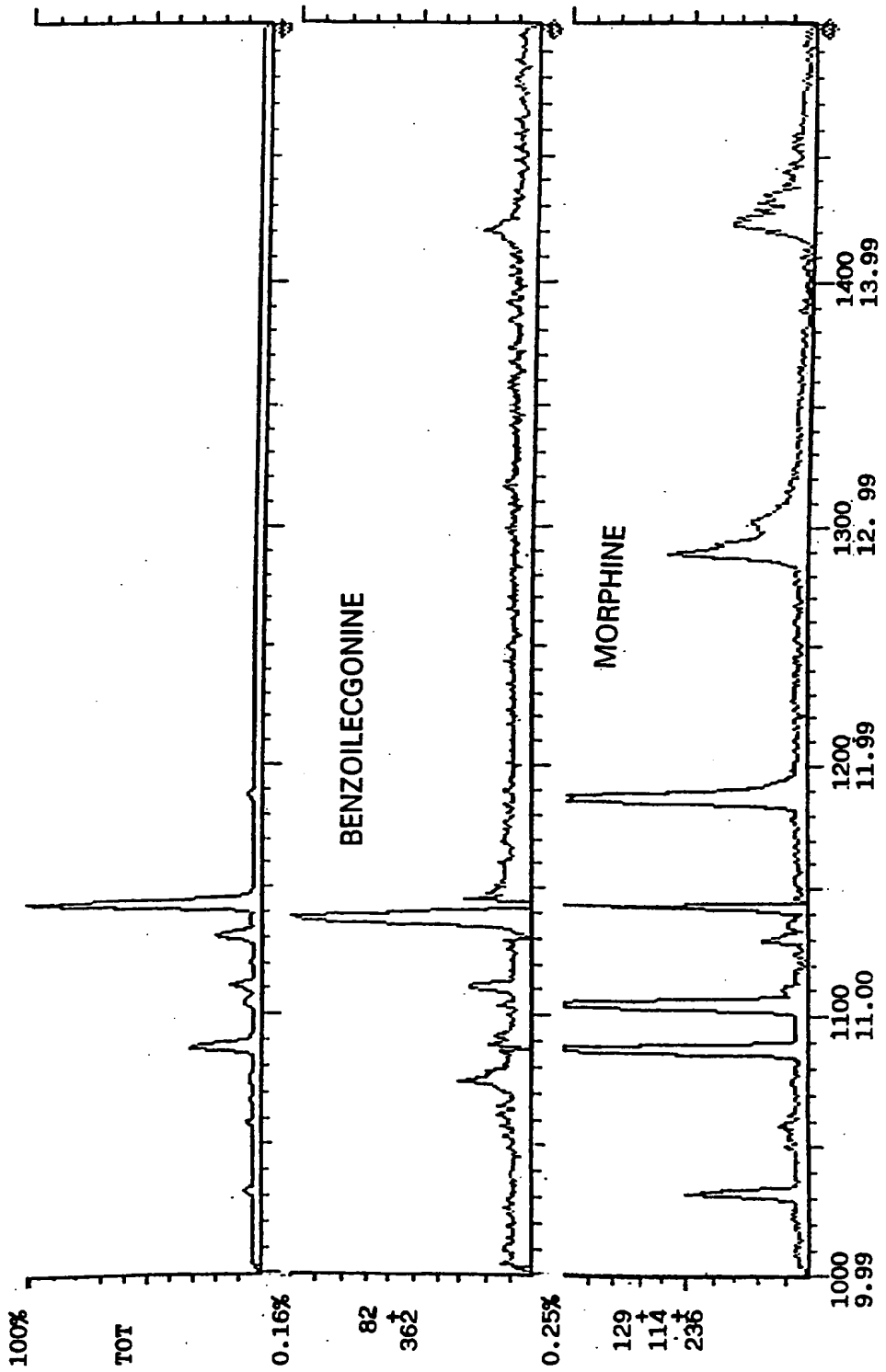

100%
TOT
0.16%
82
362
BENZOILECGONINE
0.25%
129
114
236
MORPHINE
1000
9.99
1100
11.00
1200
11.99
1300
12. 99
1400
13.99

Fig. 9

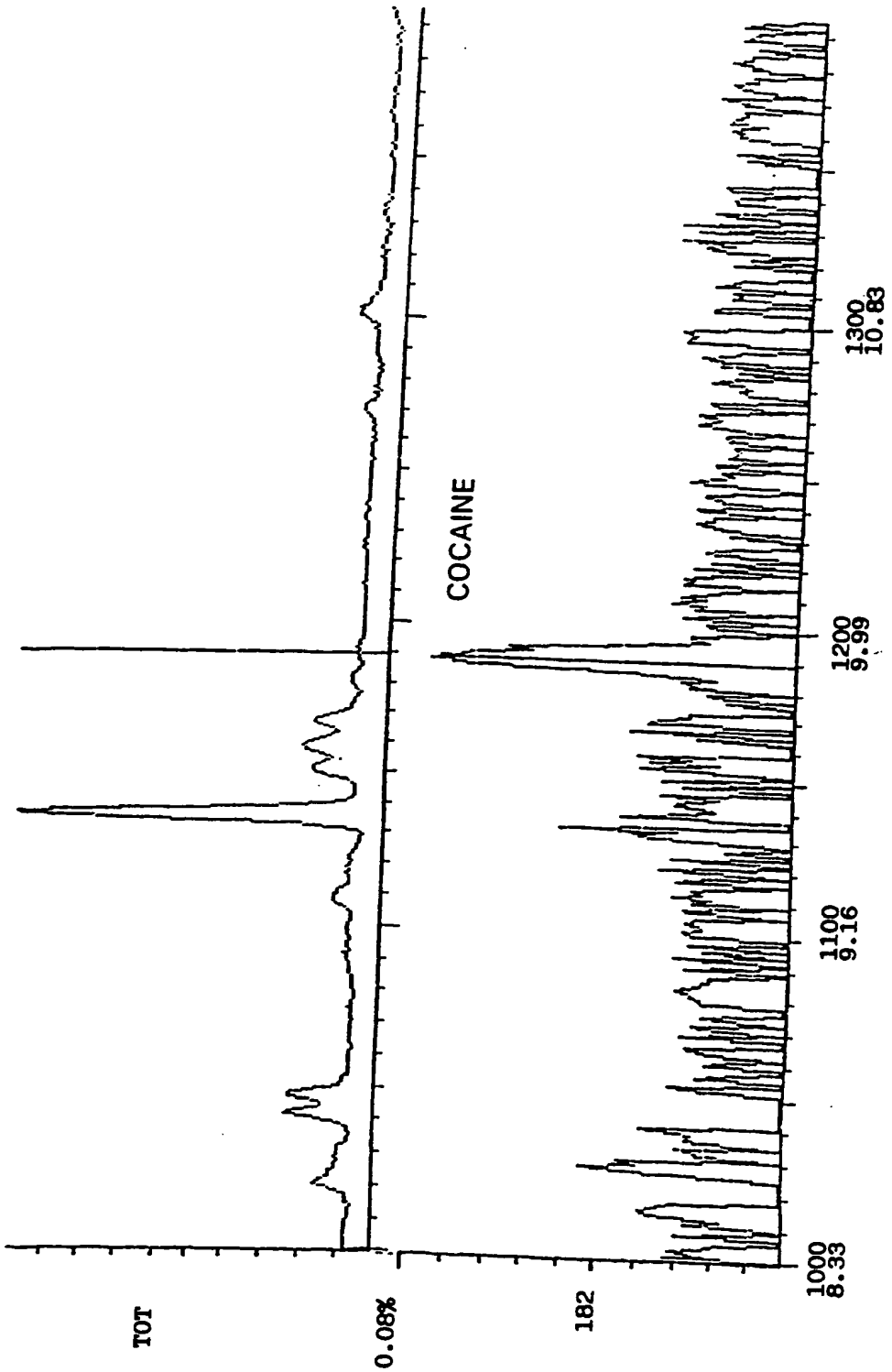


Fig. 10

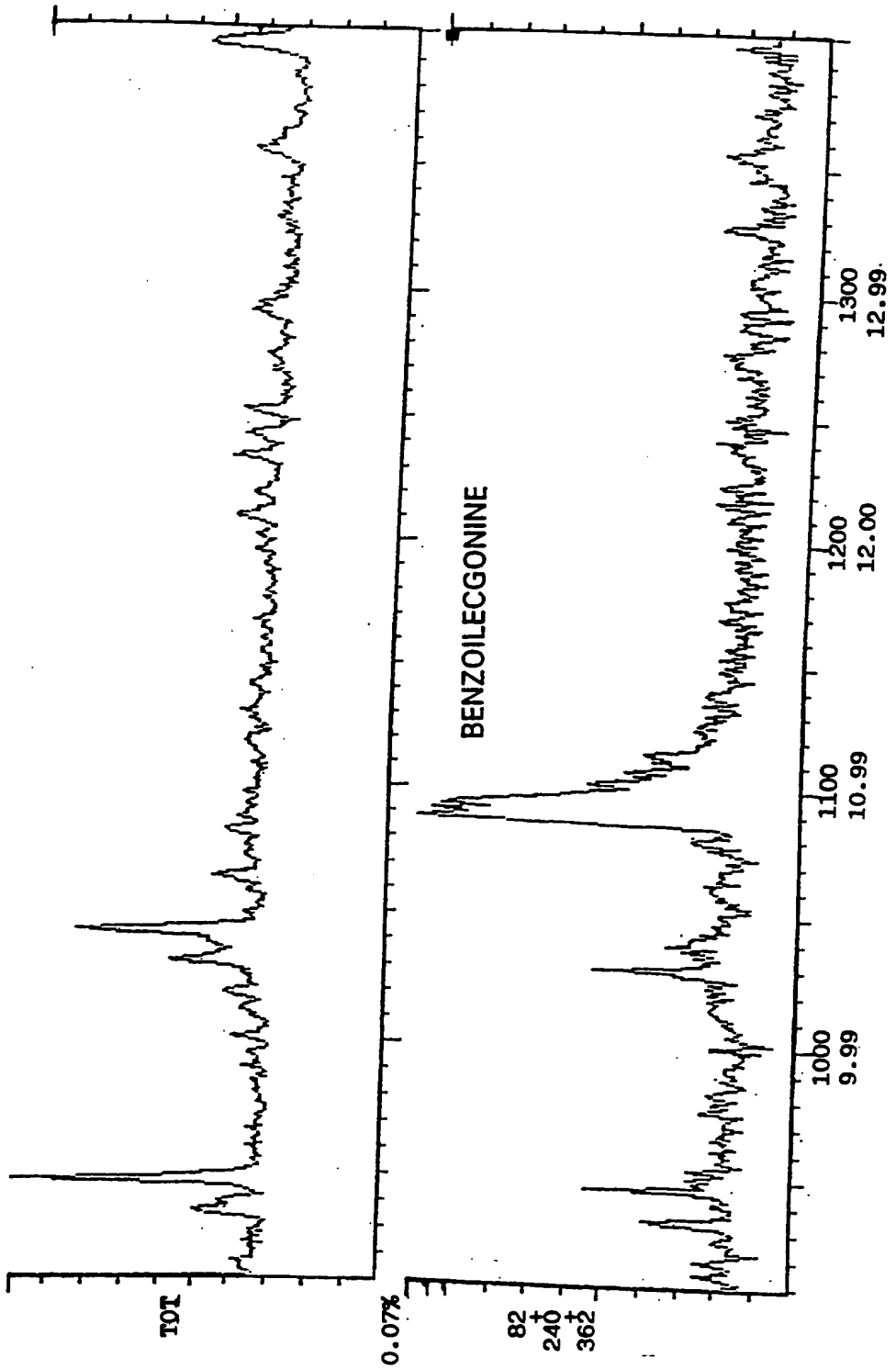

TOT
0.07%
82
240
362
BENZOILECGONINE
1000
9.99
1100
10.99
1200
12.00
1300
12.99

Fig. 11

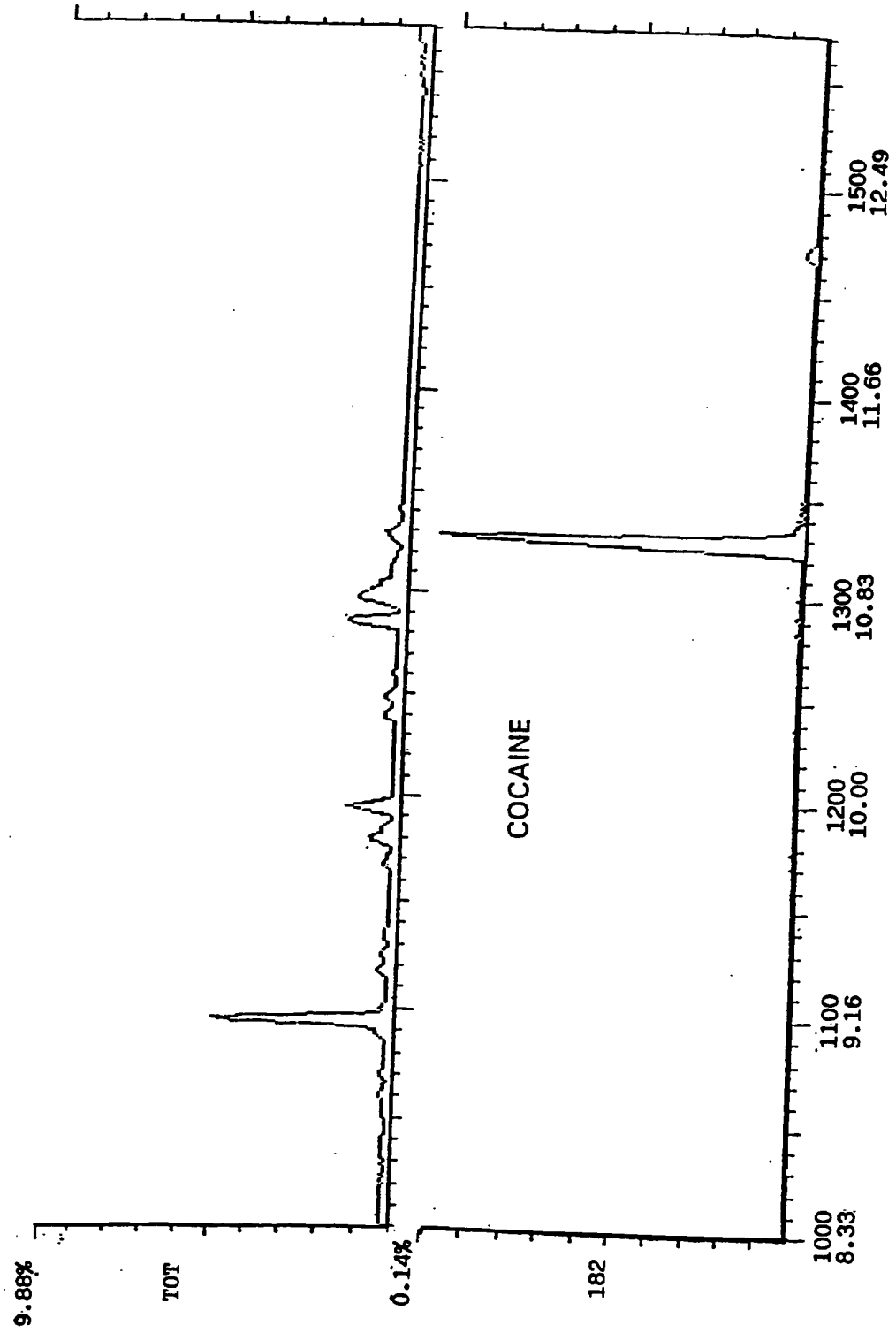

9.88%
TOT
0.14%
182
COCAINE
1000
8.33
1100
9.16
1200
10.00
1300
10.83
1400
11.66
1500
12.49

Fig. 12

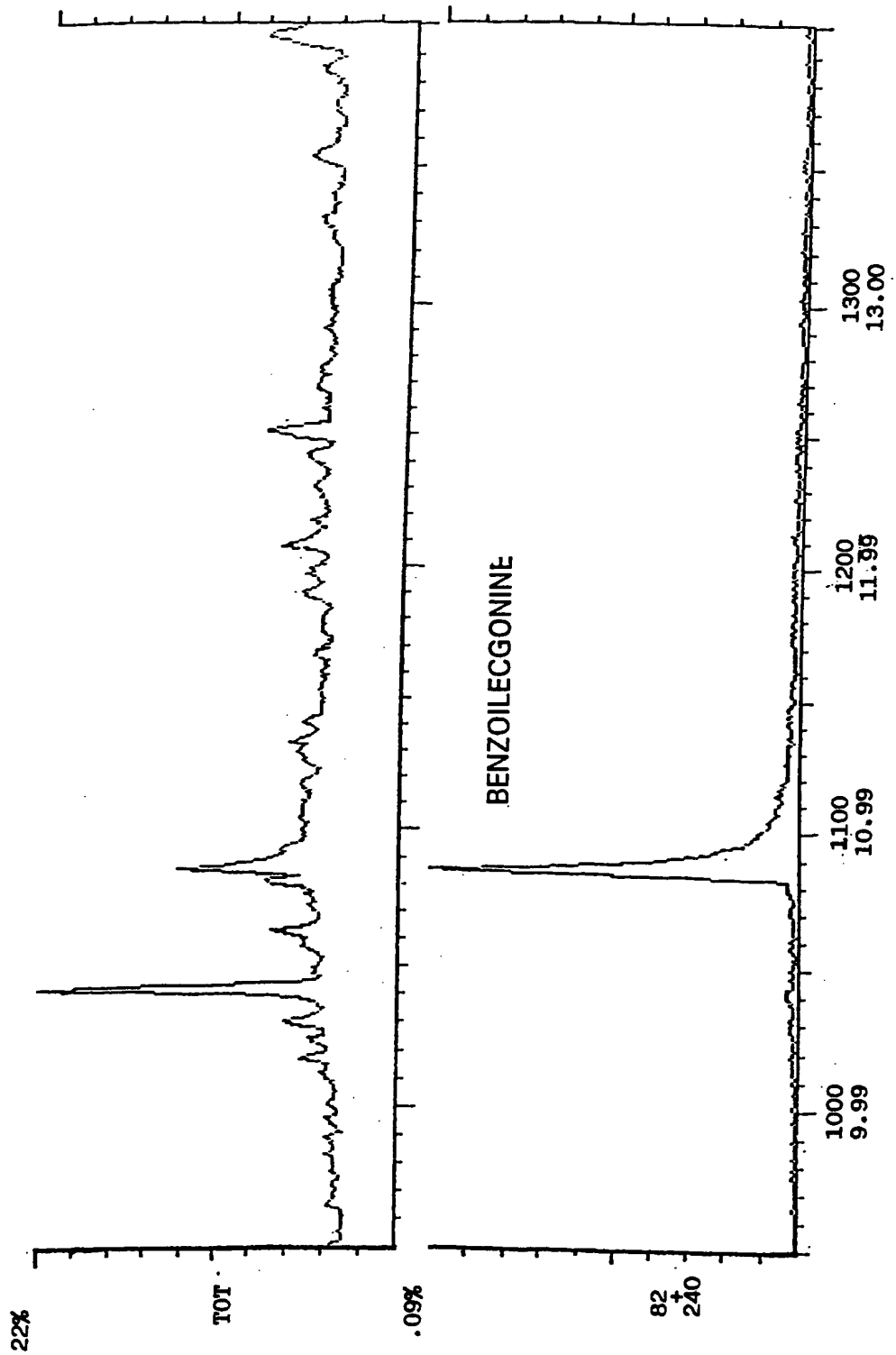

22%
TOT
.09%
82
240
BENZOILECGONINE
1000
9.99
1100
10.99
1200
11.99
1300
13.00

Fig. 13

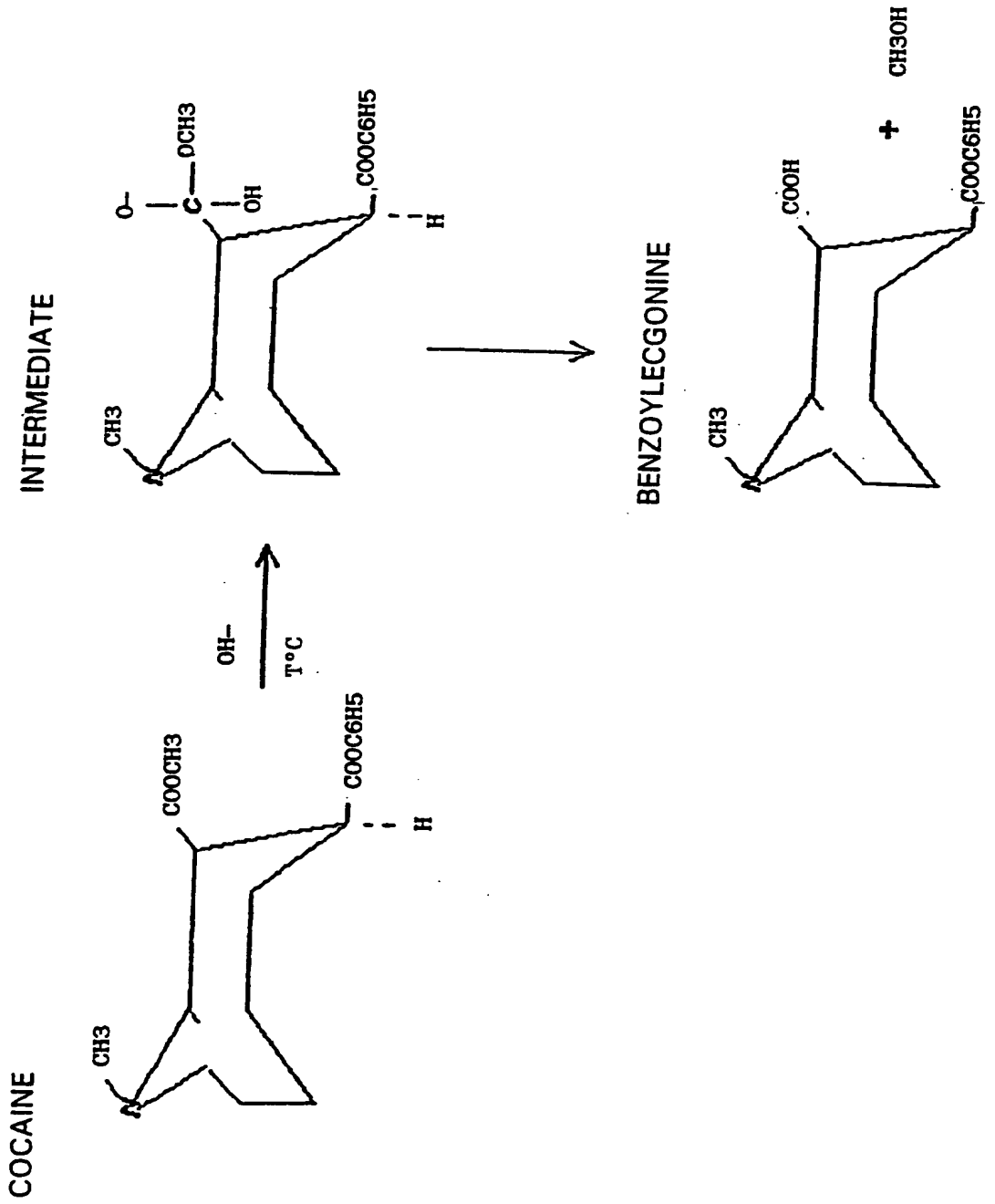

INTERMEDIATE
BENZOYLECGONINE
COCAINE
OH-
T°C
CH3OH
+
CH3
COOCH3
COOC6H5
COOH
OCH3
OH
H
O

Fig. 14

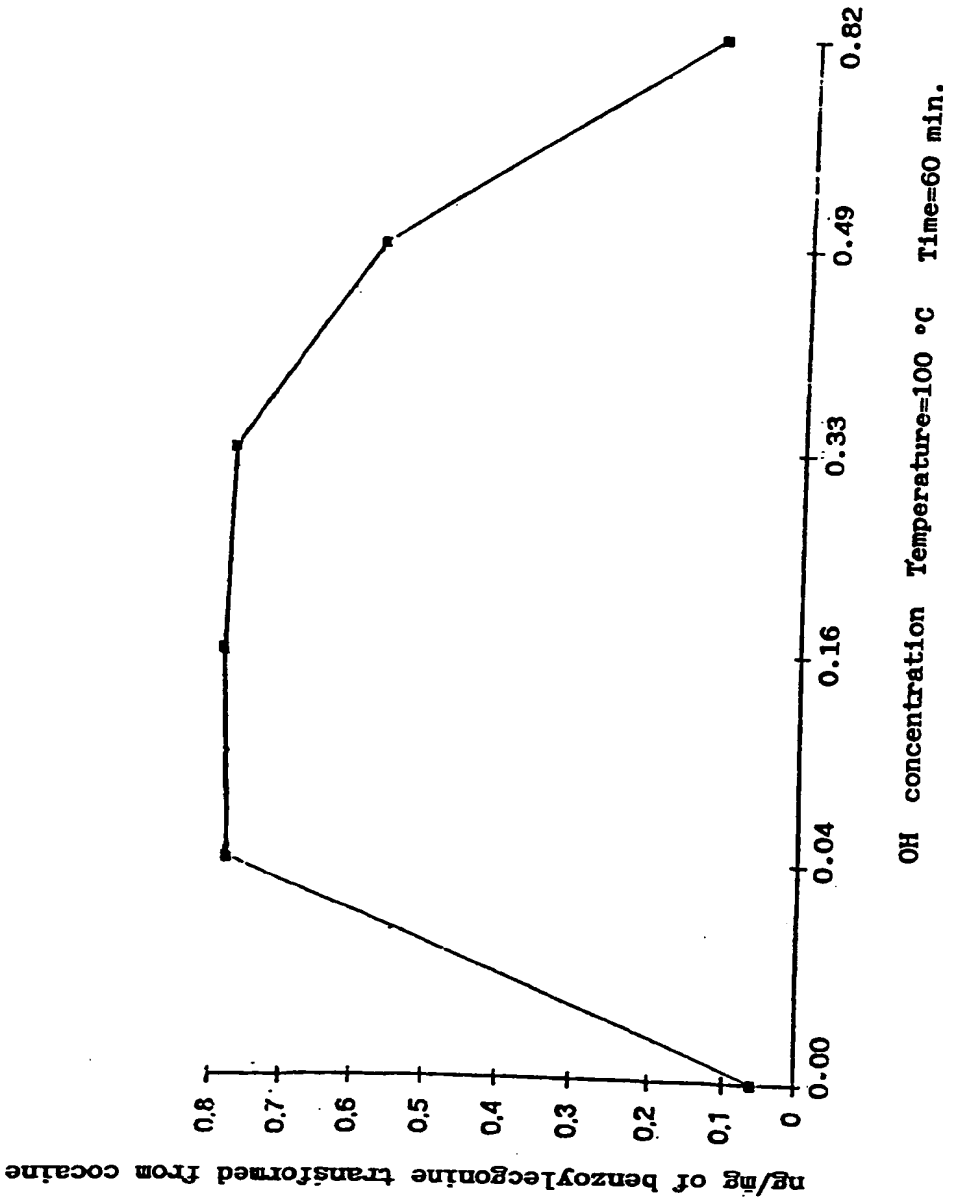

ng/mg of benzoylecgonine transformed from cocaine
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.04
0.16
0.33
0.49
0.82
OH concentration Temperature=100 °C Time=60 min.

Fig. 15

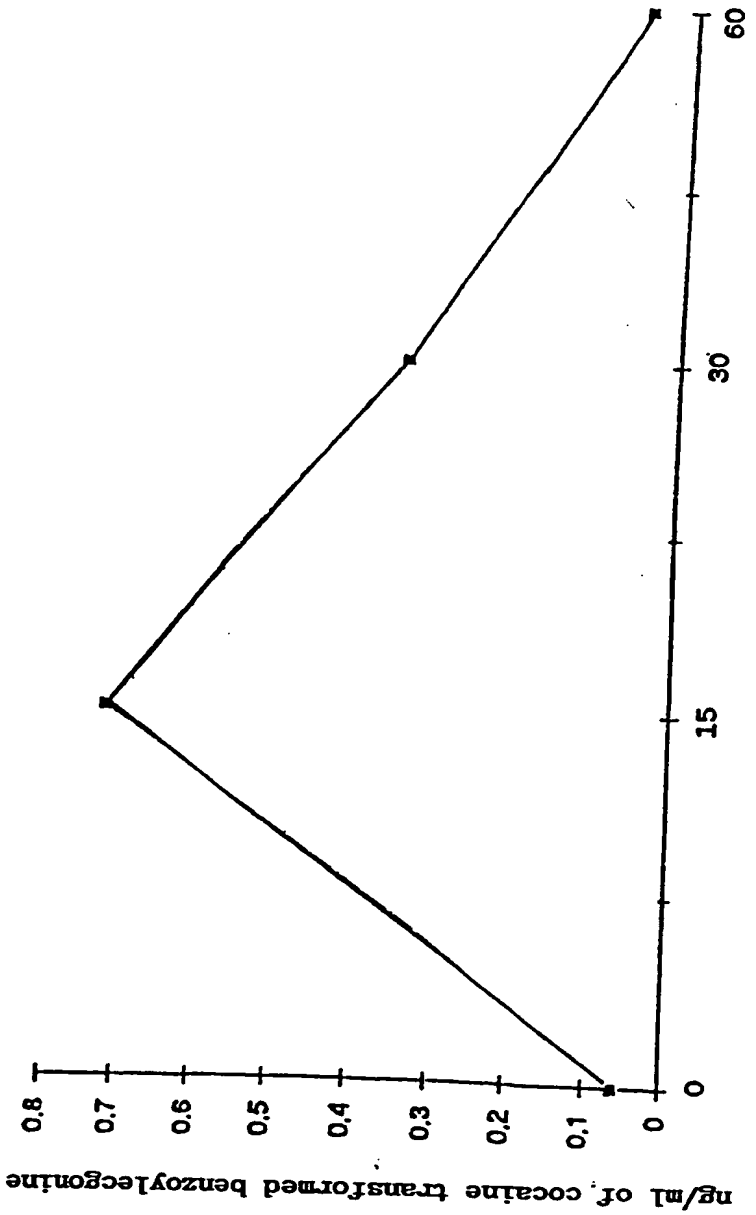

ng/ml of cocaine transformed benzoylecgonine
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
15
30
60
Temperature=150 °C

Fig. 16